Practice Laminar high Resolution fMRI Analysis

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Peters Lab pipeline

Based on Oliver Warrrington PhD Thesis

Communication of perceptual predictions from the hippocampus to the deep layers of the parahippocampal cortex

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Anatomical Preprocessing

Cortical Surface Reconstruction and Coregistration:

1. The gradient-echo image acquired at the later inversion time (INV2) volume was bias-corrected and segmented with unified segmentation method in SPM.
2. The CSF, bone, non-brain tissue and background tissue classes were combined with a threshold of > 0.5 and then inverted to create a brainmask.
3. The combined uniform (UNI) volume was denoised with the mp2rage toolbox for SPM12 with threshold of 6
4. The denoised UNI image was then skull-stripped using the INV2-derived brain mask.
5. The pial and white matter surfaces were reconstructed with CAT (input: The skull-stripped UNI)
6. The local intensity corrected output of cat12 was supplied to recon-all with the hires pipeline and samseg segmentation.
7. The cortical surfaces were then coregistered to the mean functional image using the OpenFmriAnalysis toolbox.

Anatomical Preprocessing

Cortical Surface Reconstruction and Co-registration:

8. First, the MT-weighted whole-brain EPI scan was coregistered and resliced with a rigid-body transformation to the mean functional using FSL FLIRT
9. The whole-brain EPI was then used as the target for surface coregistration due to the improved grey/white matter contrast imparted by the MT-weighting.
10. A rigid-body transformation between the UNI image and the whole-brain EPI was calculated using SPM12.
11. This transformation was then applied to the coordinates of each vertex comprising the pial and white matter surfaces, resulting in cortical surfaces in the functional space of each participant. These surfaces were then used to define the cortical layers.

Anatomical Preprocessing

Definition of the cortical layers:

1. First, The grey matter was divided into three equivolume layers using the level set method (described in detail in [66] and [67])
2. We calculated two intermediate surfaces between the WM and pial boundaries, yielding three GM layers (deep, middle, and superficial).
3. Based on these surfaces, we calculated four signed distance functions (SDF), containing for each functional voxel its distance to the boundaries between the five cortical compartments (WM, CSF, and the 3 GM layers). This set of SDFs (or "level set") allowed the calculation of the distribution of each voxel's volume over the five compartments [66].
4. For each cortical ROI (see below), voxels were assigned to one of the three GM layers only if >50% of its volume resided within that layer.

66. Van Mourik T, Van Der Eerden JPJM, Bazin P-L, Norris DG. Laminar signal extraction over extended cortical areas by means of a spatial GLM. Bernhardt BC, editor. PLOS ONE. 2019;14: e0212493. doi:10.1371/ journal.pone.0212493
67. Kok P, Bains LJ, van Mourik T, Norris DG, de Lange FP. Selective Activation of the Deep Layers of the Human Primary Visual Cortex by Top-Down Feedback. Current Biology. 2016;26: 371–376. doi:10.1016/ j.cub.2015.12.038

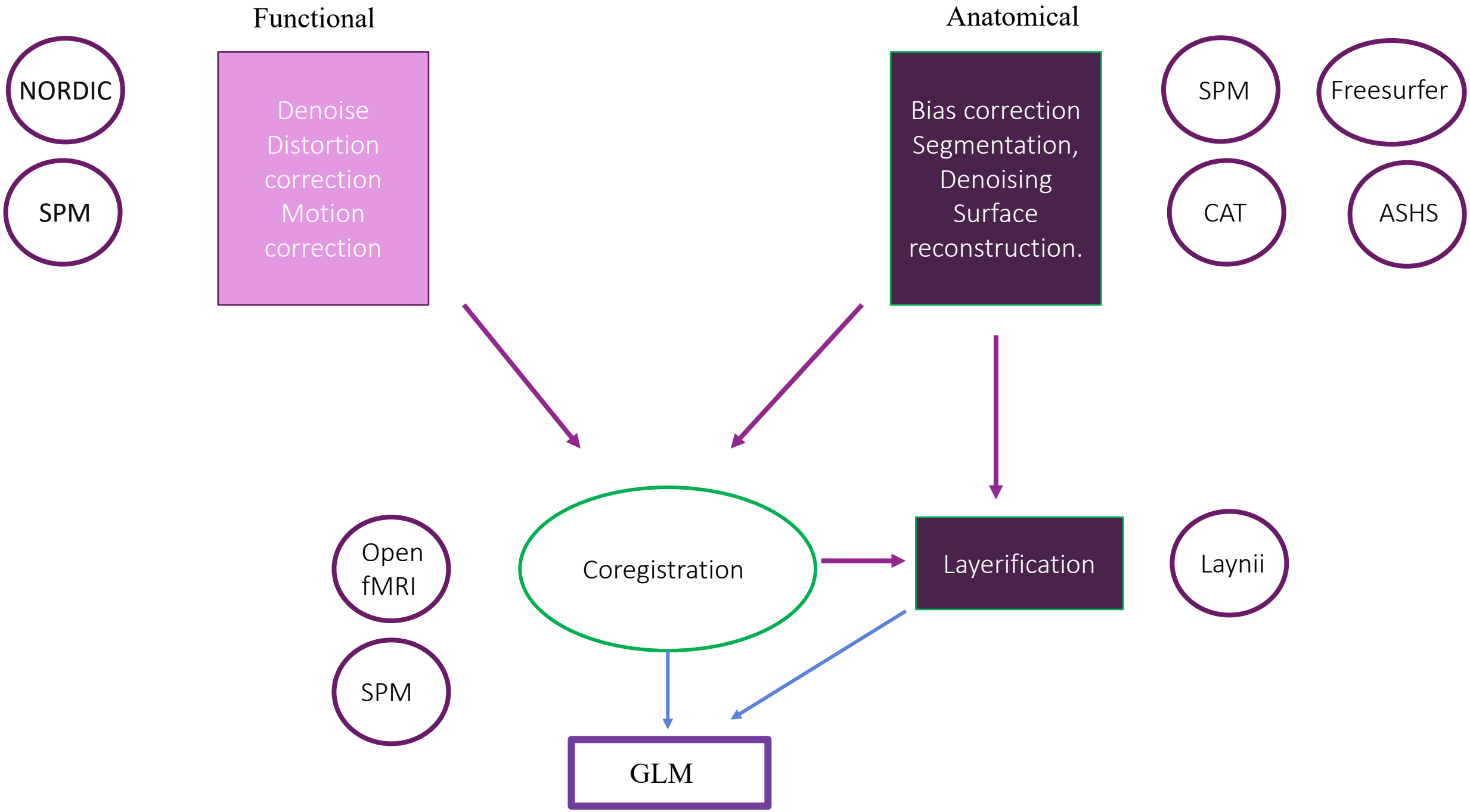
Anatomical Preprocessing

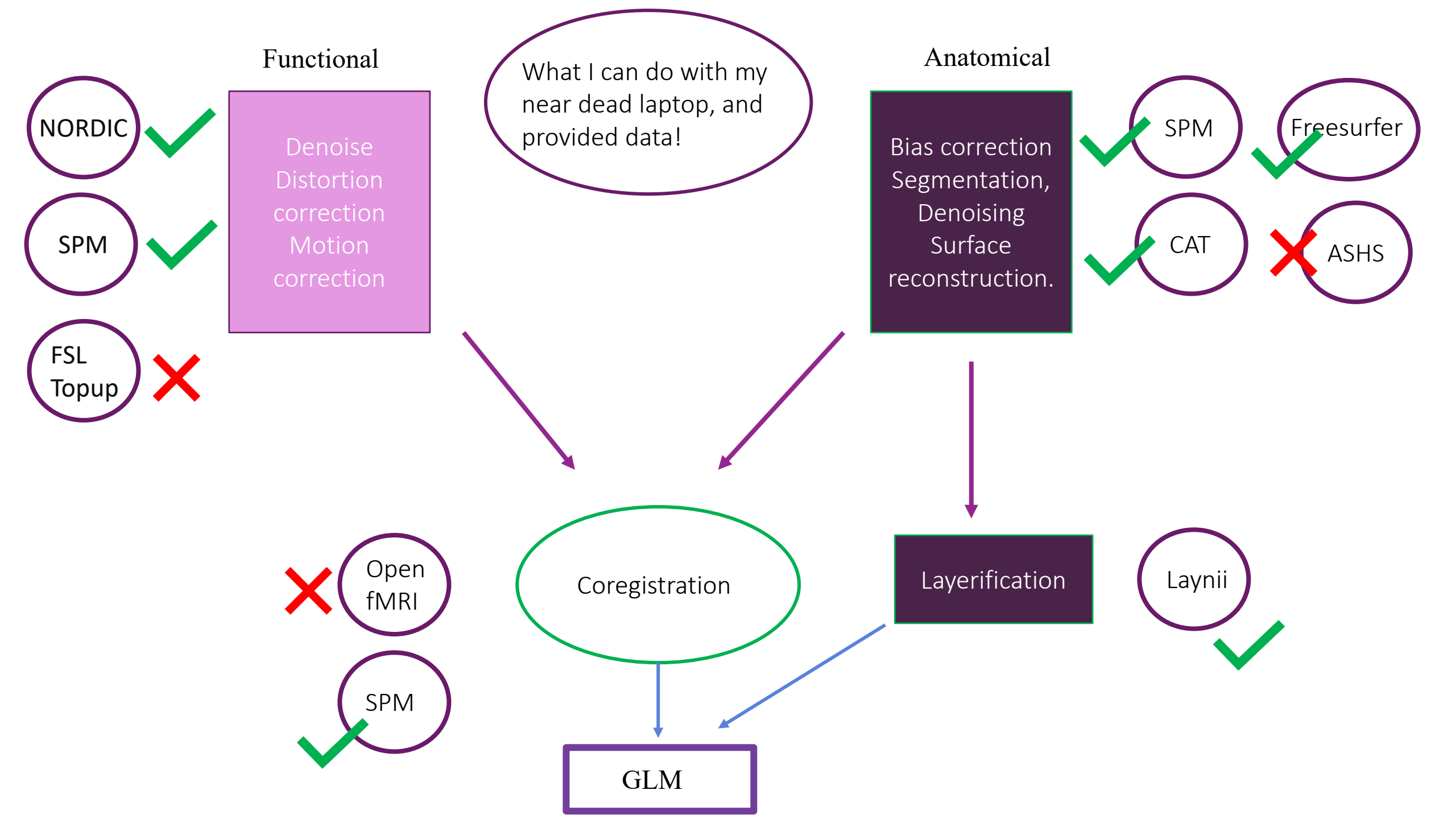
Regions of interest:

1. The medial temporal lobe was segmened using the automatic segmentation of hippocampal subfields (ASHS) [22] machine learning toolbox in conjunction with a database of manual 7T medial temporal lobe segmentations from a separate set of participants.
2. The hippocampal subfields and MTL cortices were defined on the anatomy of the T1- and T2-weighted images. The T1-weighted image was the denoised UNI volume and the T2-weighted image comprised the average of two high-resolution T2-weighted scans which were combined and denoised using the Structural Averaging Toolbox.
3. All regions segmented with ASHS were coregistered to the mean functional image of each participant using FSL FLIRT.

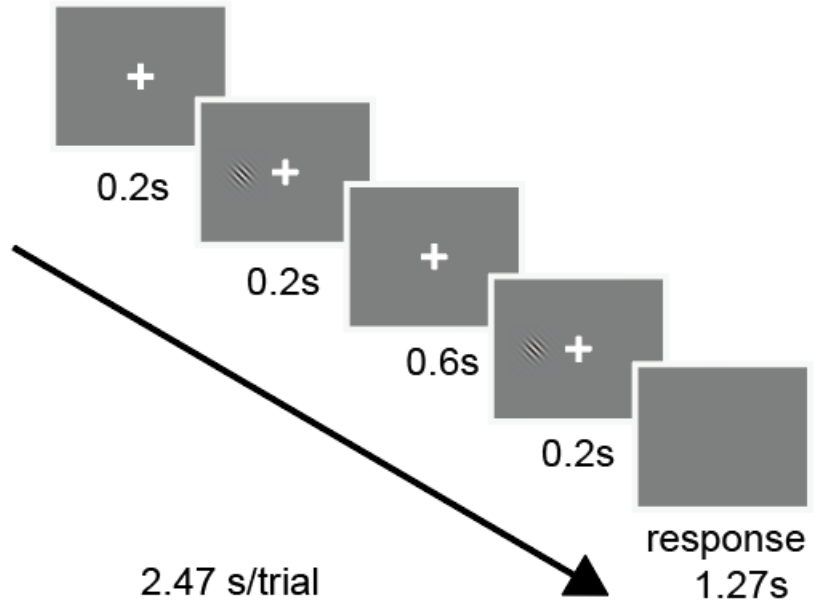
fMRI Preprocessing

1. Functional volumes denoised with NORDIC algorithm.
2. low signal slices removed.
3. Distortion correction using FSL Topup and SPM12 realign and unwarp.
4. GLM


Functional
Anatomical
NORDIC
SPM
Denoise
Distortion correction
Motion correction
Bias correction
Segmentation,
Denoising
Surface reconstruction.
SPM
Freesurfer
CAT
ASHS
Open fMRI
SPM
Coregistration
Layerification
Laynii
GLM


Functional
What I can do with my near dead laptop, and provided data!
Anatomical
NORDIC
SPM
FSL Topup
Denoise
Distortion correction
Motion correction
Bias correction
Segmentation,
Denoising
Surface reconstruction.
SPM
Freesurfer
CAT
ASHS
Open fMRI
SPM
Coregistration
Layerification
Laynii
GLM

Data from Prof. Kourtzi Lab



Session1/2/3

Run1 Run2 ..... Run10

Stimuli shown at left visual field.
Stimuli shown at right visual field.

dummy blank Ori3 Ori2 Ori1 Ori3 Ori2 Ori1 Fix .....

2TR 4TR

5 trials 12.36s (6TR)

- 7 blocks/cycle, 42TR/cycle
- 4 cycles/run, 168TR/run
- 6 TR at the beginning
- In total, 174TR/run

Data from Prof. Kourtzi Lab

	Session 1 (PL-Pre)	Session 2 (PL-Post)	Session 3 (Control)
Before EPI	0.65iso anatomical, ~35 mins	fast anatomical, ~30 mins	fast anatomical, ~30 mins
Task Runs	FDL (7.5 mins) * 10 ~80mins	FDL (7.5 mins) * 10 ~80mins	FDL (7.5 mins) * 10 ~80mins
Other runs	Resting State ~10min	Resting State ~10min	Retinotopy ~10min
In total	120 mins	120 mins	120 mins

Segmentation

1. Pre-processing (skull-Stripped UNI)
2. MP2RAGE data to Free Surfer
3. MP2RAGE data to CAT12
4. Manual Segmentation with ITK-SNAP > create rim file (after co-registeration)

MP2RAGE data to CAT12

SPM>CAT12>preprocessing>Segment>Batch(Surface and Thickness estimation: Yes, Deformation: Forward, Atlas: Yes)

CAT-Segmentation: ..analysis\training dataset_kurtzi\Shared\anatomical_highRes\zk18w7_042_brain.nii

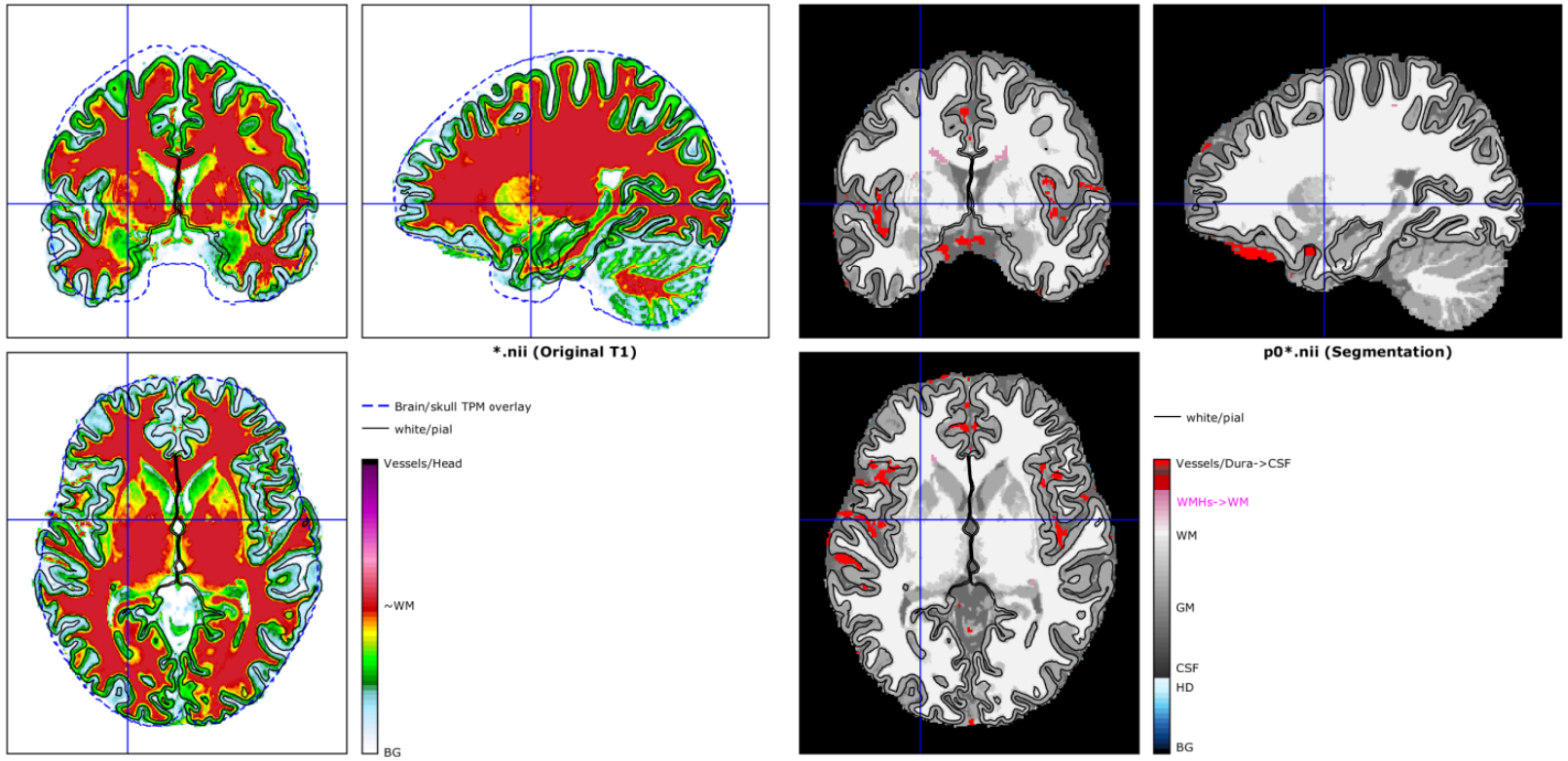
Version: OS / Matlab / SPM12 / CAT12 / seg:	WIN / 9.13 / 7771 / 12.9 (2577) / 1639
Tissue Probability Map:	\color[rgb]{0 0 0}..ers\ASUS\Desktop\Apps\spm12\tpm\TPM.nii
Optimized Shooting Registration to:	..s_MNI152NLin2009cAsym\Template_0_GS.nii
affreg / APP / setCOM	mni / default / COM
biasstr	medium
LAS strength / Skull-Stripping:	medium / APRG
WMH Correction / Int. Res.:	(WMH=WM) / optimal(1.00 0.30)
Voxel resolution (original > internal > PBT; vox):	0.65x0.65x0.65 > 0.65x0.65x0.65 > 0.50³ mm³; 1.50³ mm³

Image and Preprocessing Quality:

Resolution:	**92.00% (A-)**
Noise:	**100.00% (A+)**
Bias:	**74.72% (C)**
Weighted average (IQR):	**93.08% (A-)**
Mean surface Euler number:	40
Defect area:	1.26%
Processing time:	52:38 min

Volumes:	CSF	GM	WM
Absolute volume:	220	593	478 cm³
Relative volume:	17.0	45.9	37.0 %
TIV:	1290 cm³		
Thickness:	1.95±0.52 mm		

MP2RAGE data to CAT12: for surfaces



FreeSurfer 7.1.1: for segmentation

Code:
recon-all -subject test-subject -i ~/ zk18w7_042_brain.nii.gz –all

For outputs:

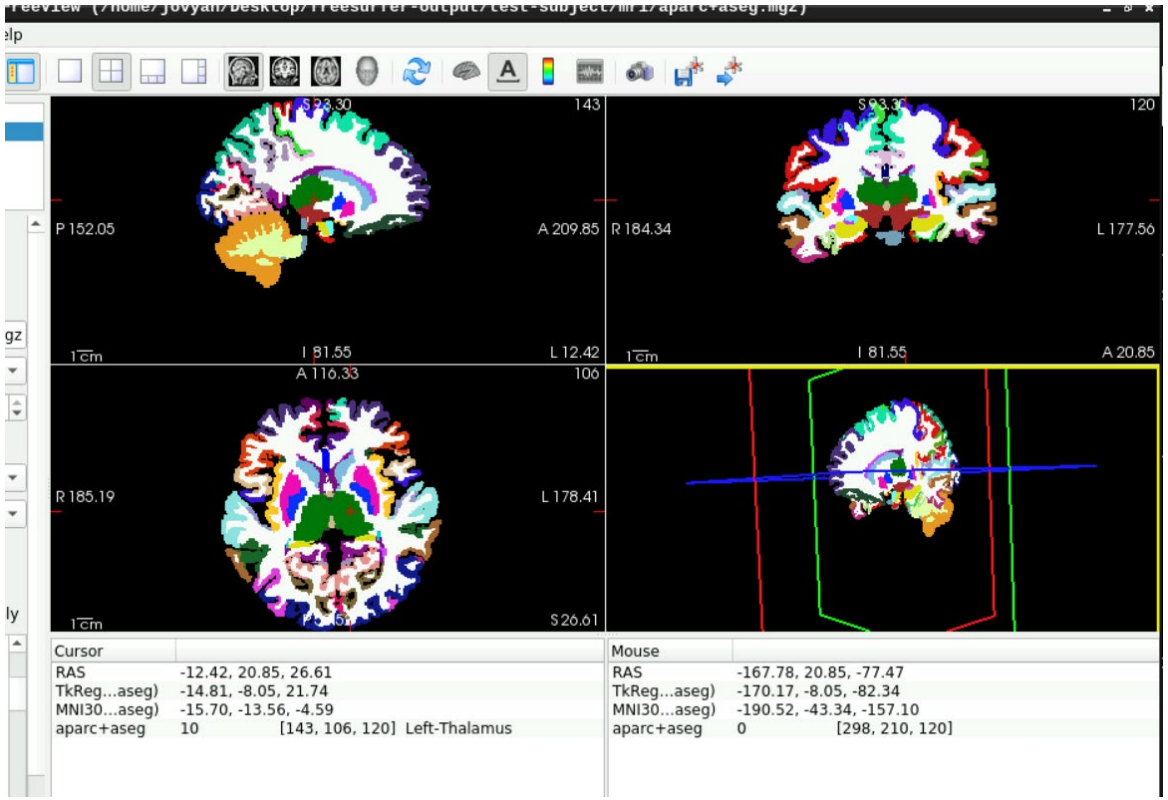
mri_label2vol –seg ribbon.mgz –temp rawavg.mgz –o ribbon-in-rawavg.mgz –regheader ribbon.mgz

mri_convert ribbon-in-rawavg.mgz ribbon-in-rawavg.nii

mri_convert rawavg.mgz rawavg.nii

FreeSurfer 7.1.1

Aparak + aseg



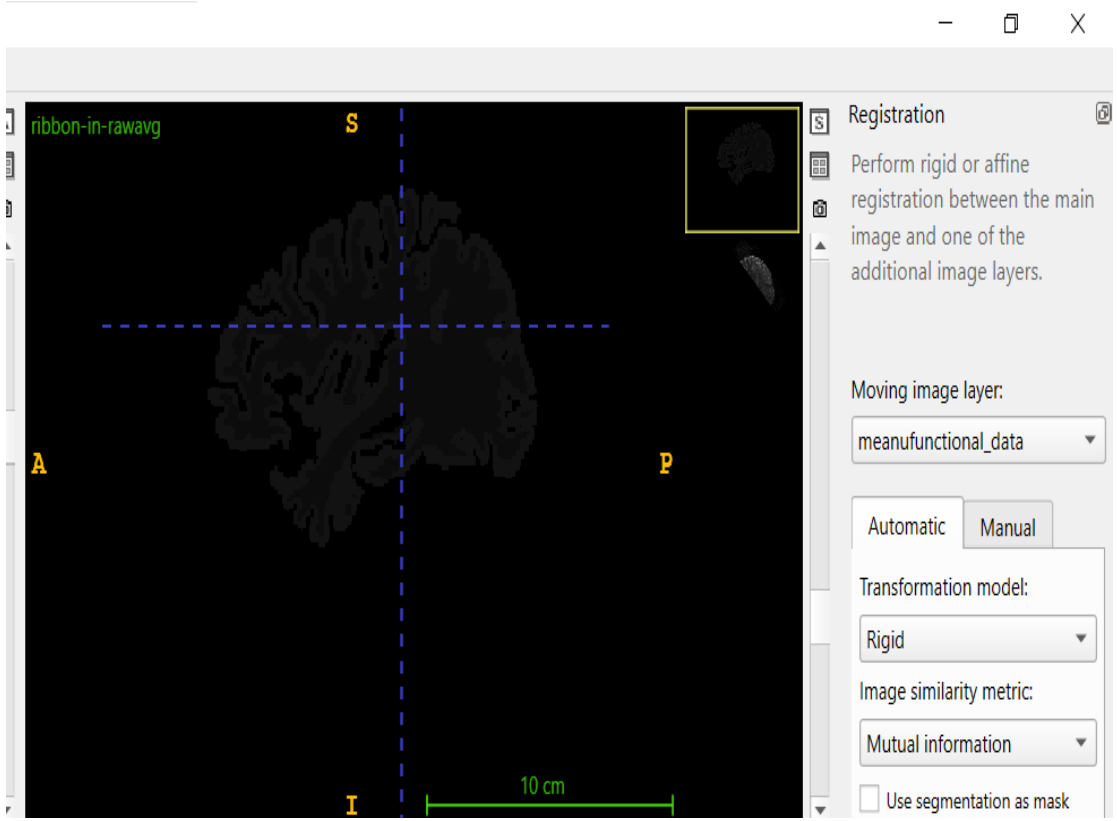
Co-registration with SPM (pial and white matter surface)

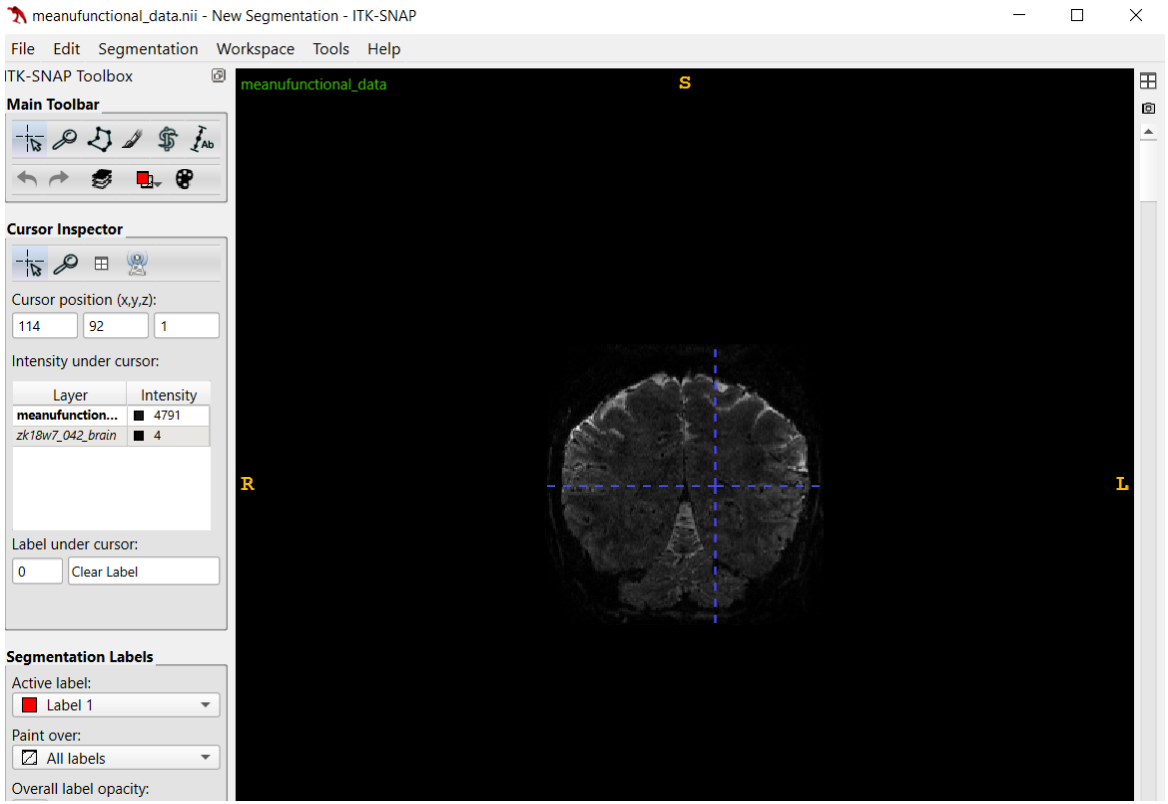
```
spm('defaults', 'FMRI');

spm_jobman('initcfg');

% Paths

mean_func = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/meanufunctional_data.nii'; % mean functional image

t1_image = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/ribbon-in-rawavg.nii'; % segmented output from freesurfer
% Coregister T1 to mean functional image

matlabbatch{1}.spm.spatial.coreg.estimate.ref = {mean_func};

matlabbatch{1}.spm.spatial.coreg.estimate.source = {t1_image};

spm_jobman('run', matlabbatch);

% Load transformation matrix

transformation_matrix = spm_get_space(t1_image);

% Surface files

lh_pial = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/lh.pial.zk18w7_042_brain.gii';

rh_pial = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/rh.pial.zk18w7_042_brain.gii';

lh_white = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/lh.white.zk18w7_042_brain.gii';

rh_white = 'D:/high field fMRI analysis/training dataset_kurtzi/Shared/co_register/rh.white.zk18w7_042_brain.gii';
```

Co-registration with SPM (pial and white matter surface)

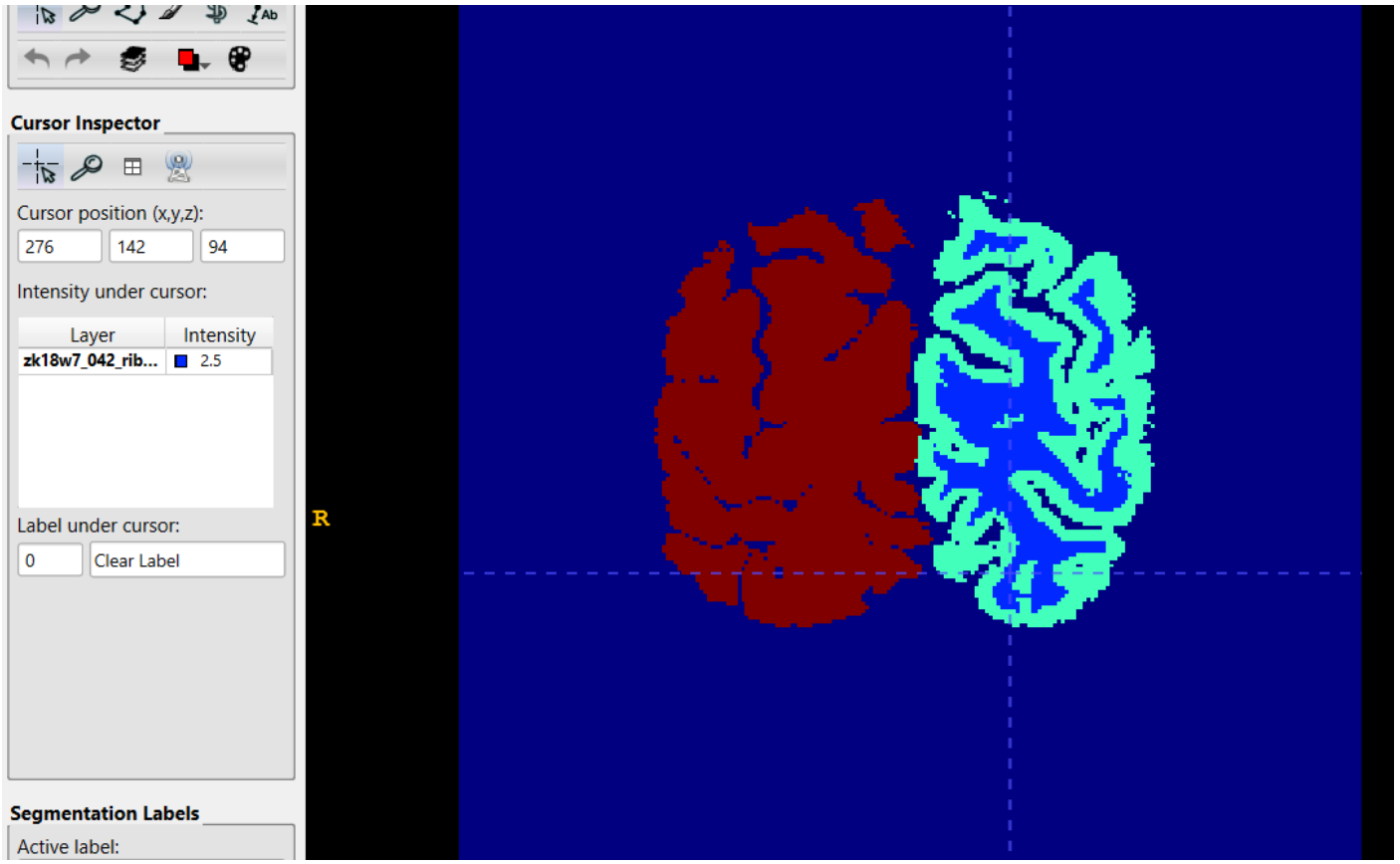
```
% Apply transformation to surfaces
apply_transformation_to_surface(lh_pial , transformation_matrix);
apply_transformation_to_surface(rh_pial , transformation_matrix);
apply_transformation_to_surface(lh_white, transformation_matrix);
apply_transformation_to_surface(rh_white, transformation_matrix);

function apply_transformation_to_surface(surface_file, transformation_matrix)
% Load the surface file
surf = gifti(surface_file);
% Apply the transformation matrix to the vertices
surf.vertices = (transformation_matrix(1:3, 1:3) * surf.vertices' + transformation_matrix(1:3, 4))';
% Save the transformed surface
save(surf, surface_file);
end
```

Check co-registration manually with ITK-SNAP



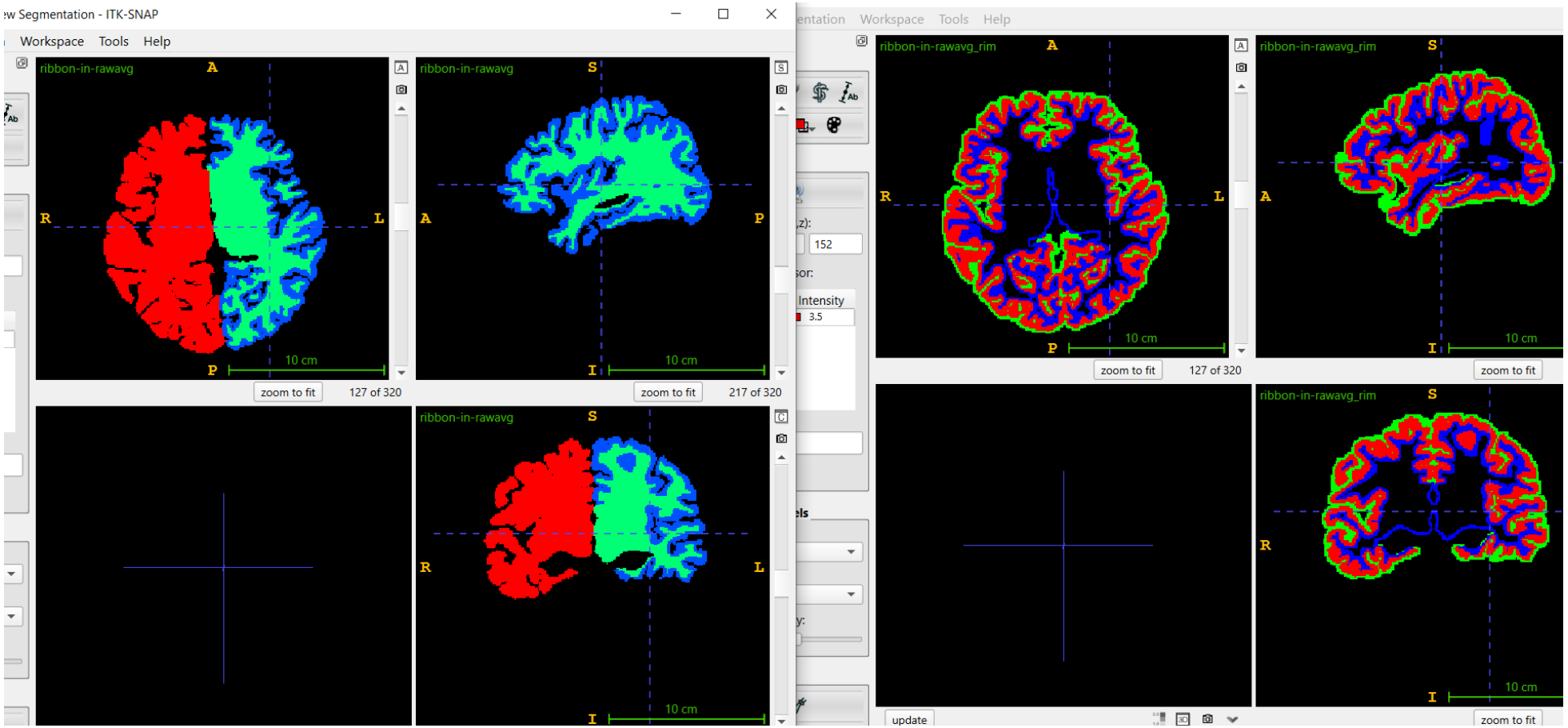


Further Manual Segmentation with ITK-SNAP



Change Labels (transfer free surfer output to rim file for laynii)

Code: https://github.com/ofgulban/LAYNII_extras/blob/38e607edbd6601b5893e519af3f791059fbe190d/demo freesurfer_segmentation_to_rim/freesurfer_segmentation_to_rim.py



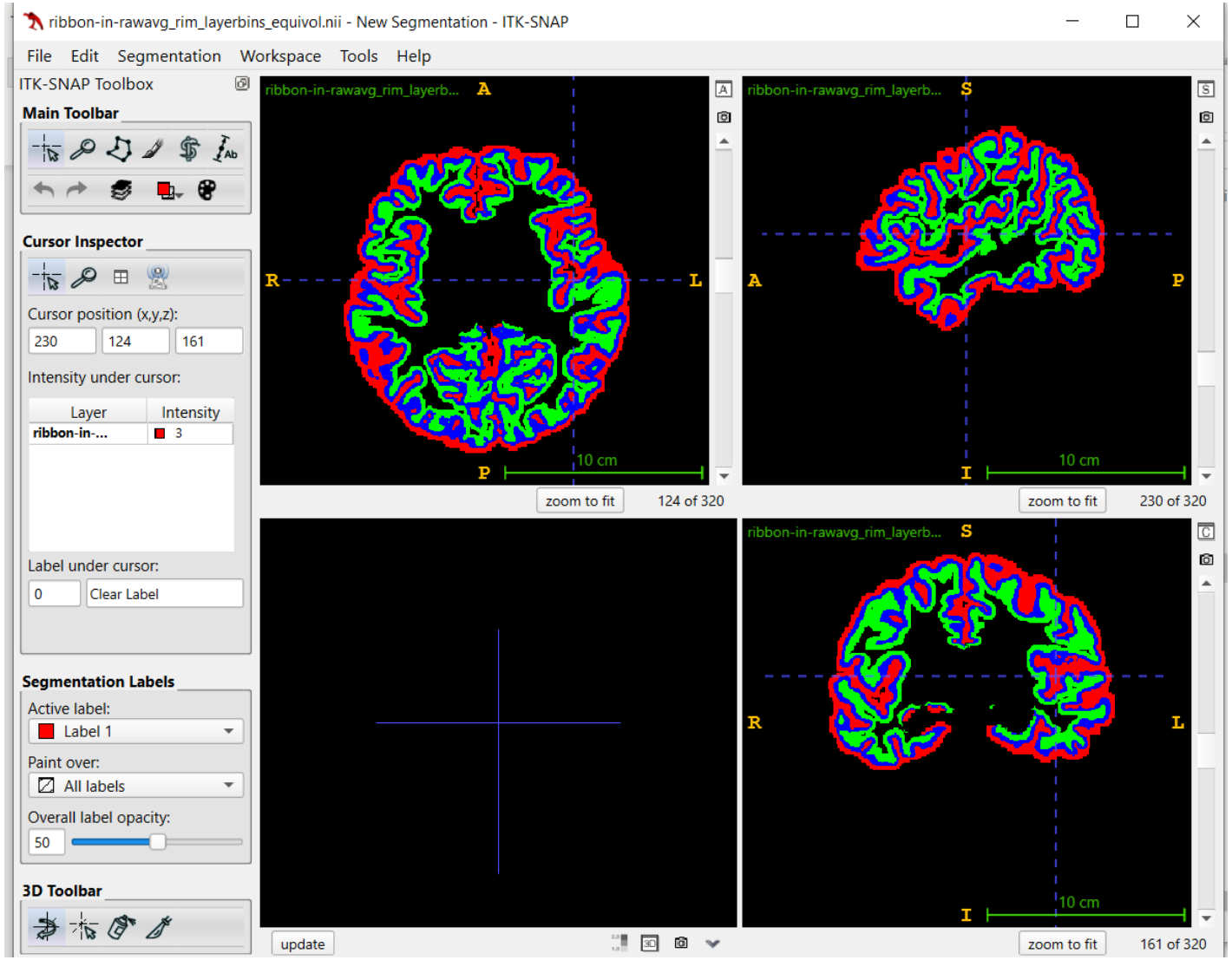
Segmentation with ASHS

I couldn’t find a suitable atlas for my data. Lets keep going with rim file.

Defining Cortical Layers in LYNII (level set method)

Code :

```
LN2_LAYERS -rim ribbon-in-rawavg_rim.nii -nr_layers 3 -equal_counts -equivol
```



Functional data

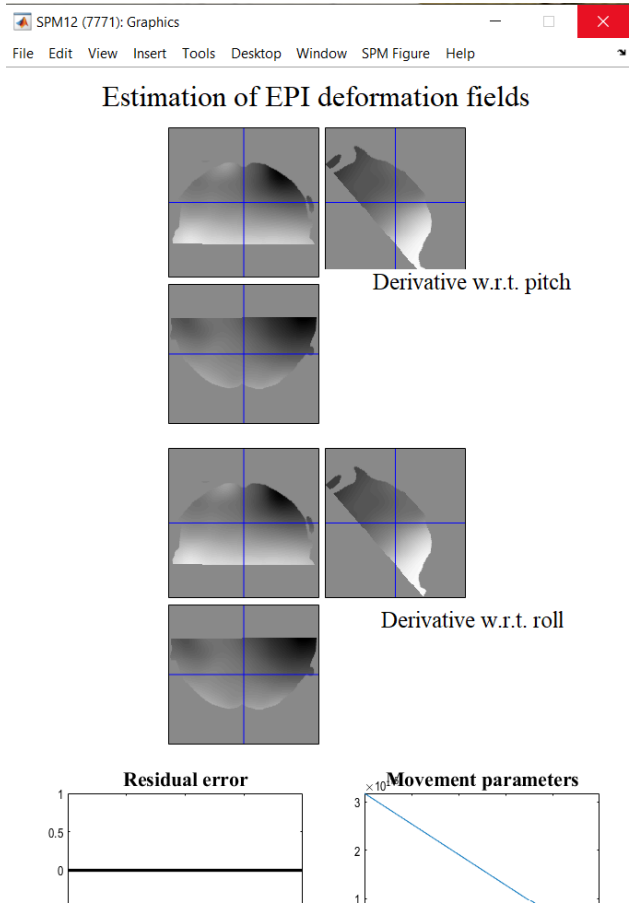
1. Create 4D nifty with SPM
2. Creating mean image with SPM
3. realign and unwarp for motion and distortion correction (not possible; I couldn't use ph hp phase)
4. Quality check with Lynii (tSNR)
5. Using NORDIC for denoising
6. Quality check again

Functional data

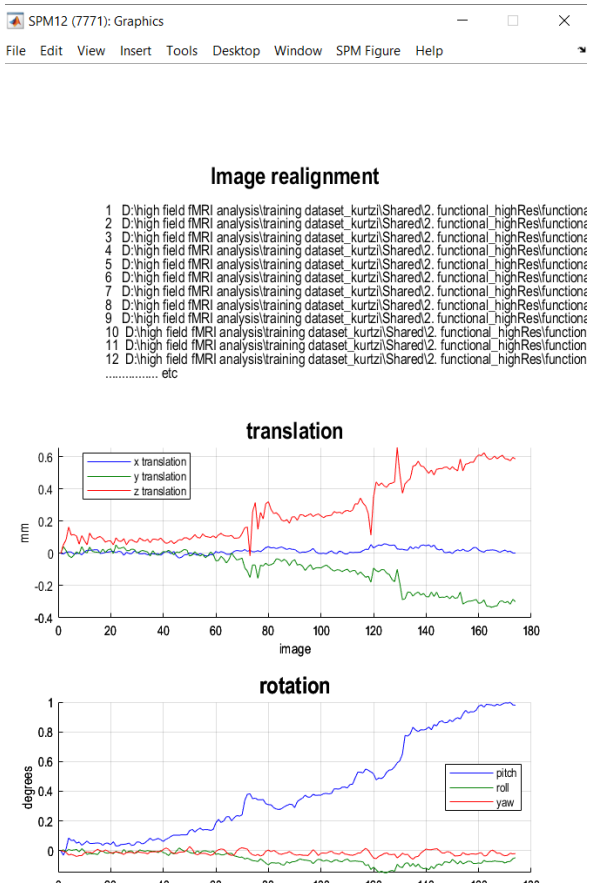
7. Slice time correction (skipped)
8. Co-registeration with OpenFmriAnalysis toolbox: (not possible)
9. Co-registeration with SPM toolbox and ITK-SNAP
10. Create V1 mask after Layerification
11. Activation within layers of V1

Realign and unwarp for motion and distortion correction

Realign and unwrap (I have no such a phase map file)



Realign (Estimate and resliced)



NORDIC denoising

Code:

v1.1 from https://github.com/SteenMoeller/NORDIC_Raw/releases

```
ARG.NORDIC=1;
RG.noise_volume_last = 0;
ARG.magnitude_only=1;
%ARG.kernel_size_PCA = [28, 28, 1];
fn_magn_in= ‘rfunctional_data';
fn_phase_in=fn_magn_in;
fn_out=['NORDIC_' fn_magn_in];
NIFTI_NORDIC(fn_magn_in,fn_phase_in,fn_out,ARG)
```

Quality Check (slice time correction skipped)

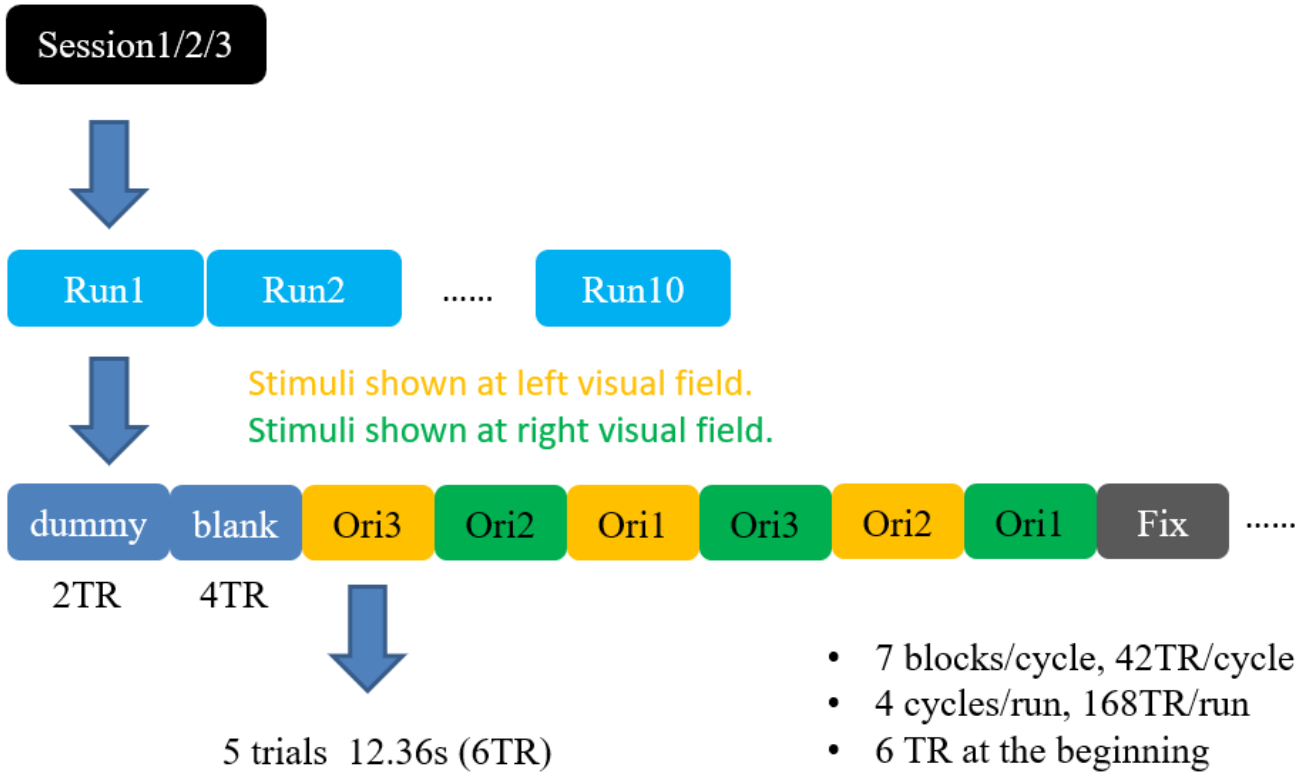
I want to see changes after NORDIC denoising

Code:

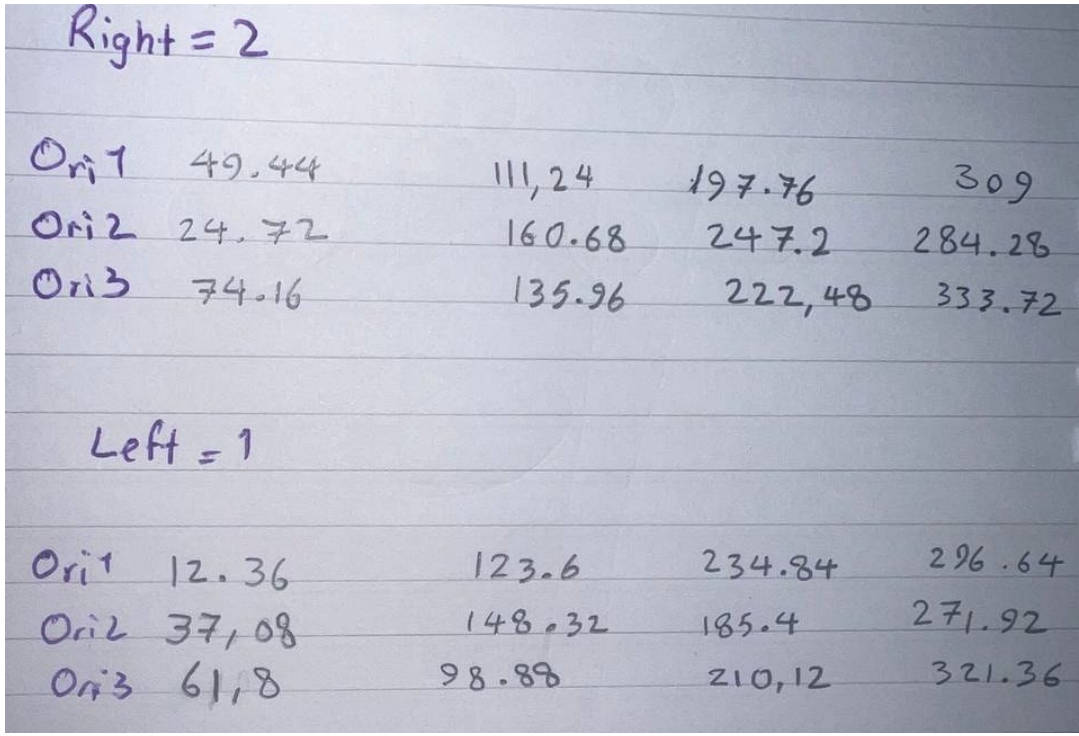
1. LN_SKEW -input NORDIC_rfunctional_data.nii
2. LN_SKEW -input rfunctional_data.nii
3. GLM with Afnii:

```
3dDeconvolve -overwrite -jobs 16 -polort a -input NORDIC_rfunctional_data.nii \
        -num_stimts 3 \
        -TR_times 2.06 \
        -stim_times 1 '1D:  49.44 111.24 197.76 309' 'UBLOCK(12.36,1)' -stim_label 1 Task1 \
        -stim_times 2 '1D: 24.72 160.68 247.2 284.28' 'UBLOCK(12.36,1)' -stim_label 2 Task2 \
        -stim_times 3 '1D: 74.16 135.96 222.48 333.72' 'UBLOCK(12.36,1)' -stim_label 3 Task3 \
        -tout \
        -iresp 1 HRF_BOLD.nii \
        -x1D MODEL_wm \
        -bucket STATS.nii


# participant's response (orientation 1-2-3), I just model for right hemisphere
```

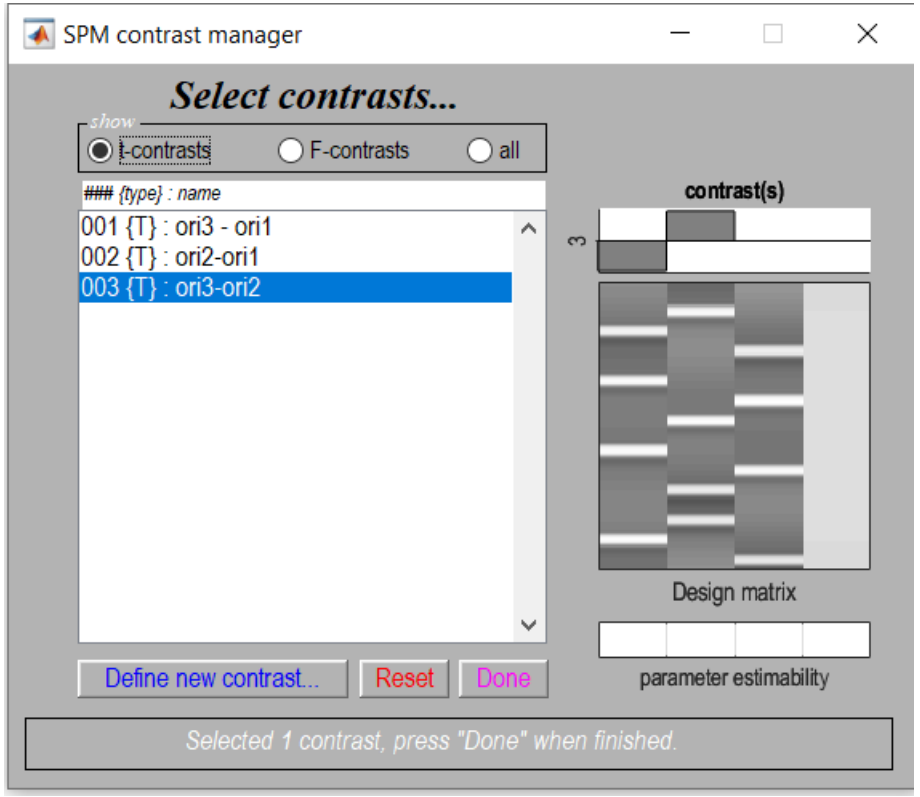
GLM (onset, duration)

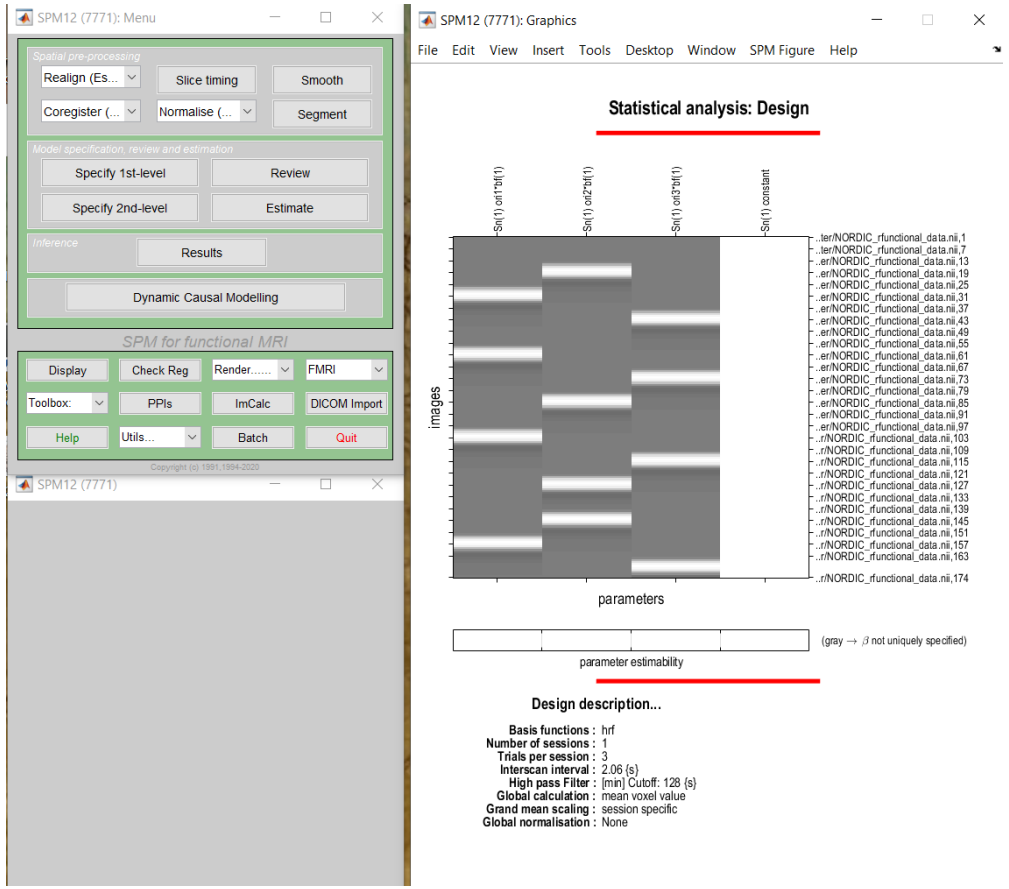


- 7 blocks/cycle, 42TR/cycle
- 4 cycles/run, 168TR/run
- 6 TR at the beginning
- In total, 174TR/run



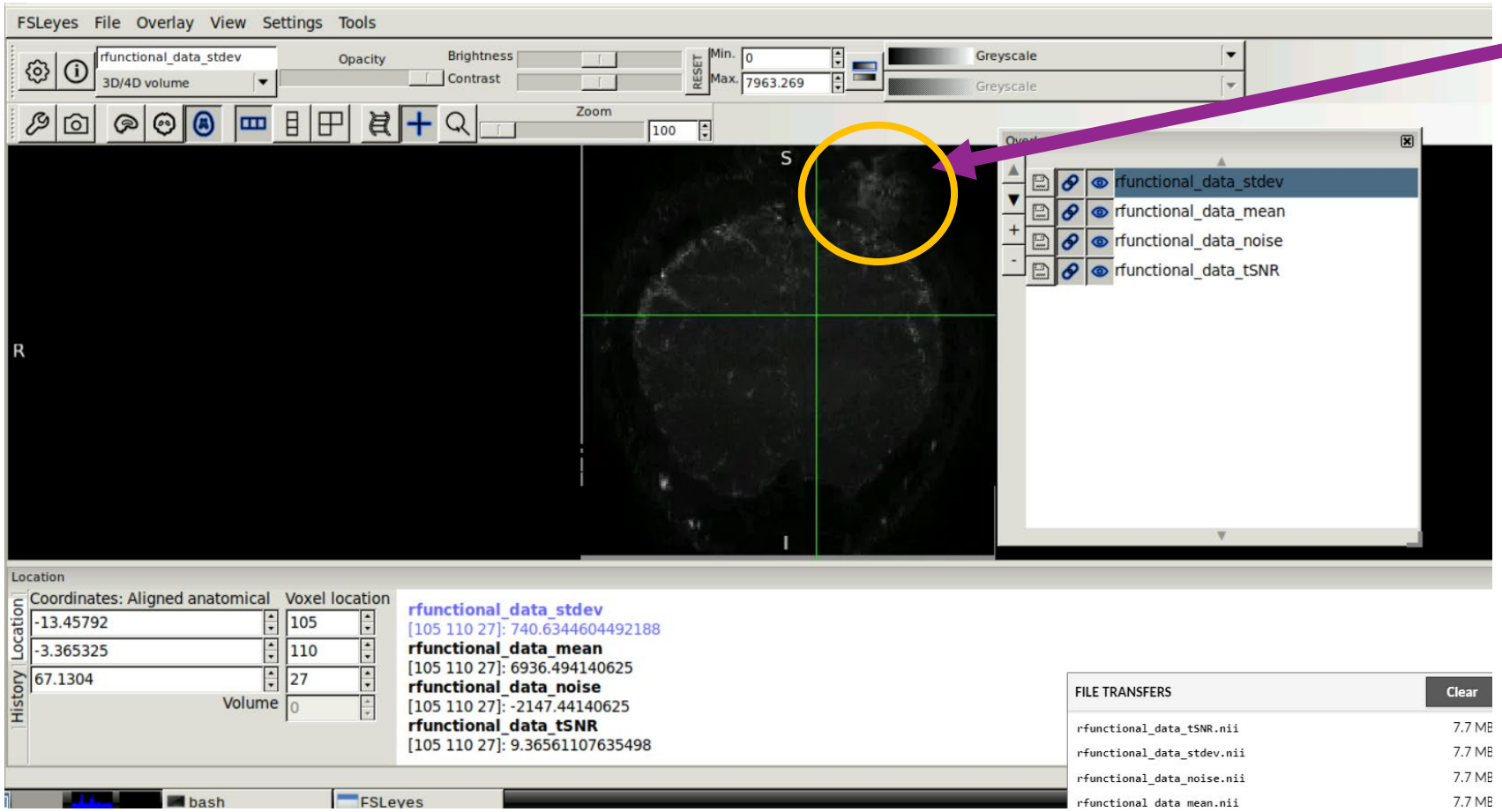
GLM with SPM


SPM contrast manager
Select contrasts...
show:
t-contrasts
F-contrasts
all
{type} : name
001 {T} : ori3 - ori1
002 {T} : ori2-ori1
003 {T} : ori3-ori2
contrast(s)
3
Design matrix
parameter estimability
Define new contrast...
Reset
Done
Selected 1 contrast, press "Done" when finished.

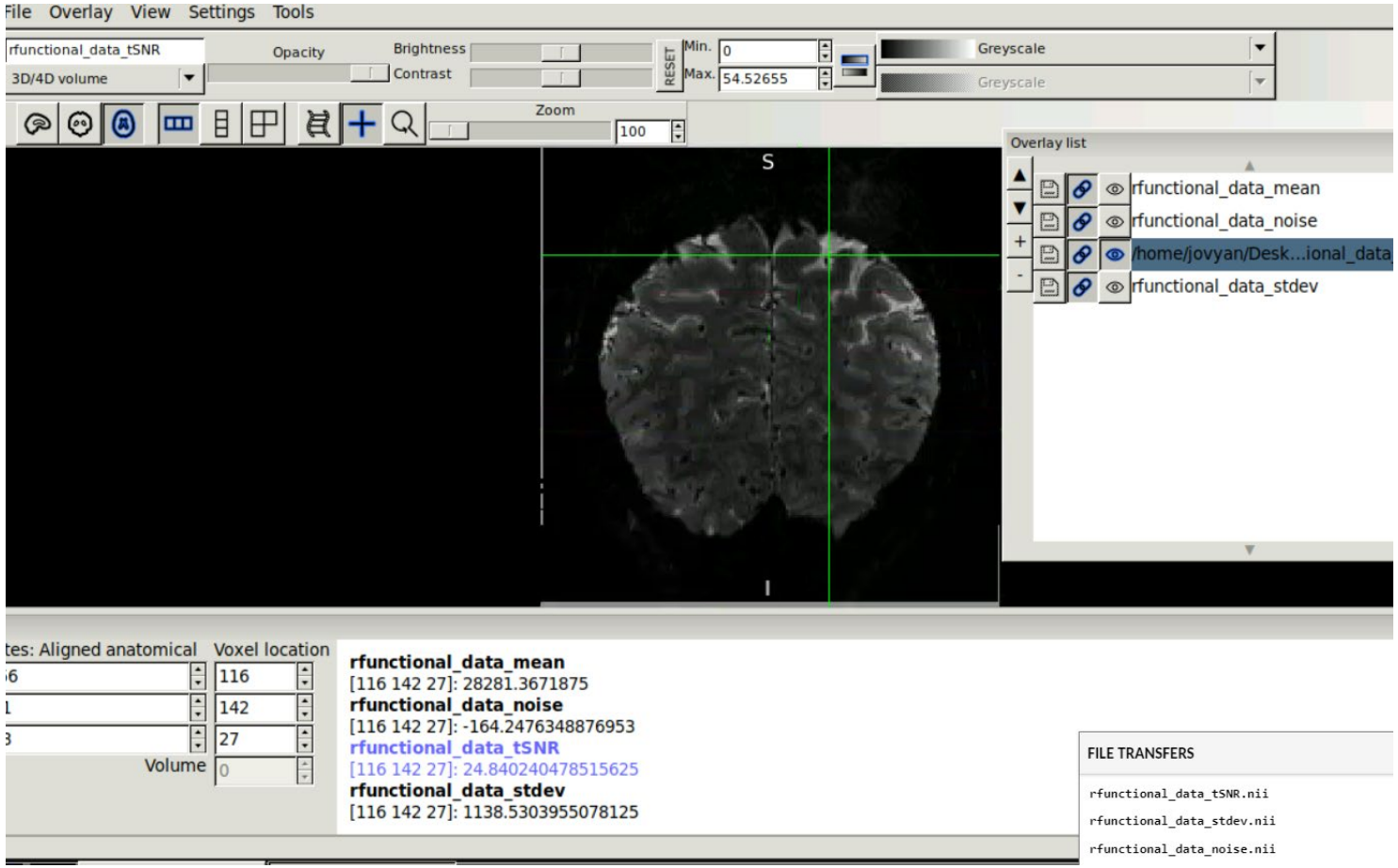

SPM12 (7771): Menu
Spatial pre-processing
Realign (Es...
Slice timing
Smooth
Coregister (...
Normalise (...
Segment
Model specification, review and estimation
Specify 1st-level
Review
Specify 2nd-level
Estimate
Inference
Results
Dynamic Causal Modelling
SPM for functional MRI
Display
Check Reg
Render.....
FMRI
Toolbox:
PPIs
ImCalc
DICOM Import
Help
Utils...
Batch
Quit
SPM12 (7771)
SPM12 (7771): Graphics
File Edit View Insert Tools Desktop Window SPM Figure Help
Statistical analysis: Design
Sn(1) ori1*bf(1)
Sn(1) ori2*bf(1)
Sn(1) ori3*bf(1)
Sn(1) constant
images
parameters
parameter estimability
(gray → β not uniquely specified)
Design description...
Basis functions : hrf
Number of sessions : 1
Trials per session : 3
Interscan interval : 2.06 (s)
High pass Filter : [min] Cutoff: 128 (s)
Global calculation : mean voxel value
Grand mean scaling : session specific
Global normalisation : None

Quality Check (no NORDIC)

Like ghost in stdev

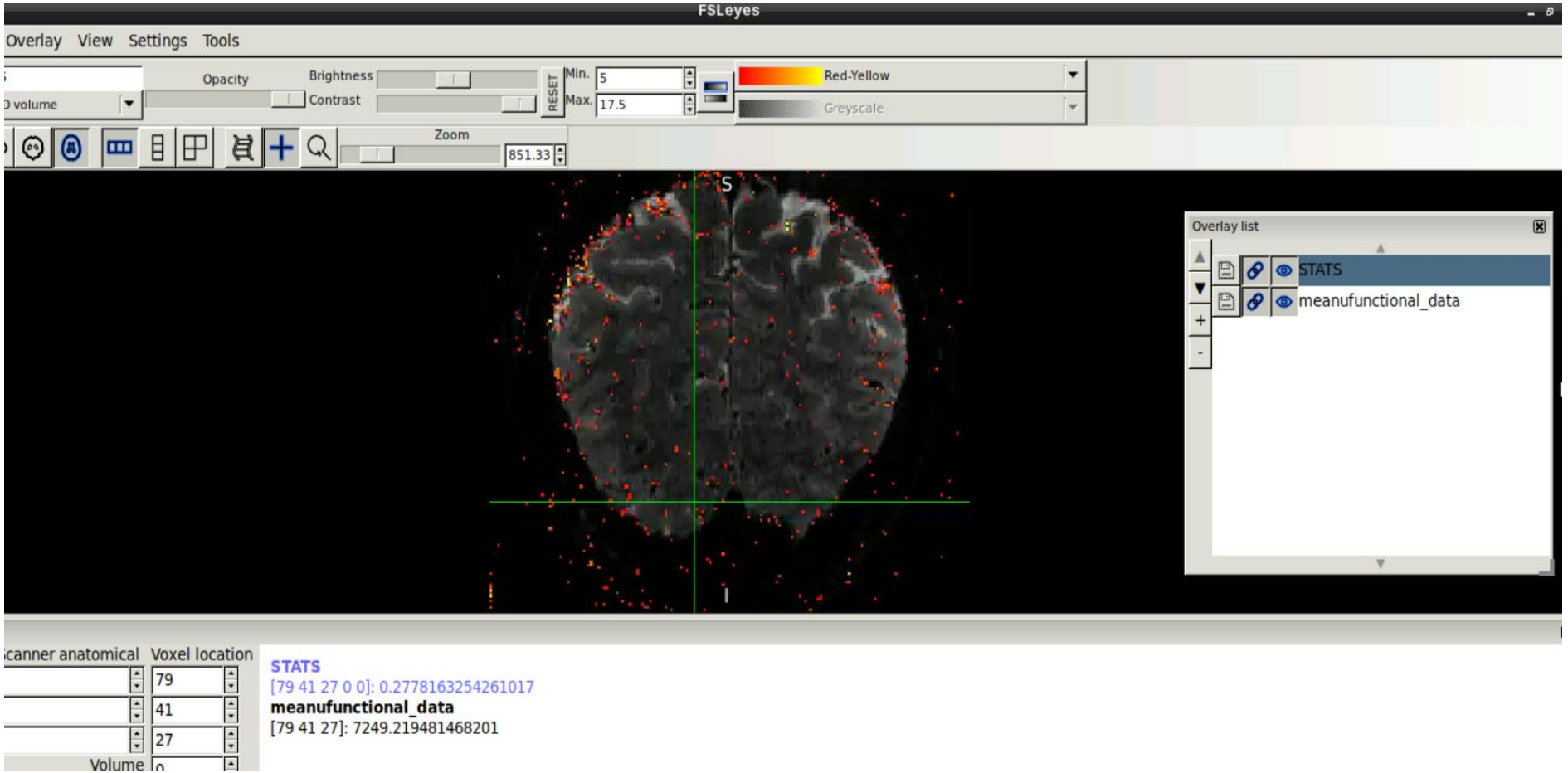


Quality Check (no NORDIC) tSNR



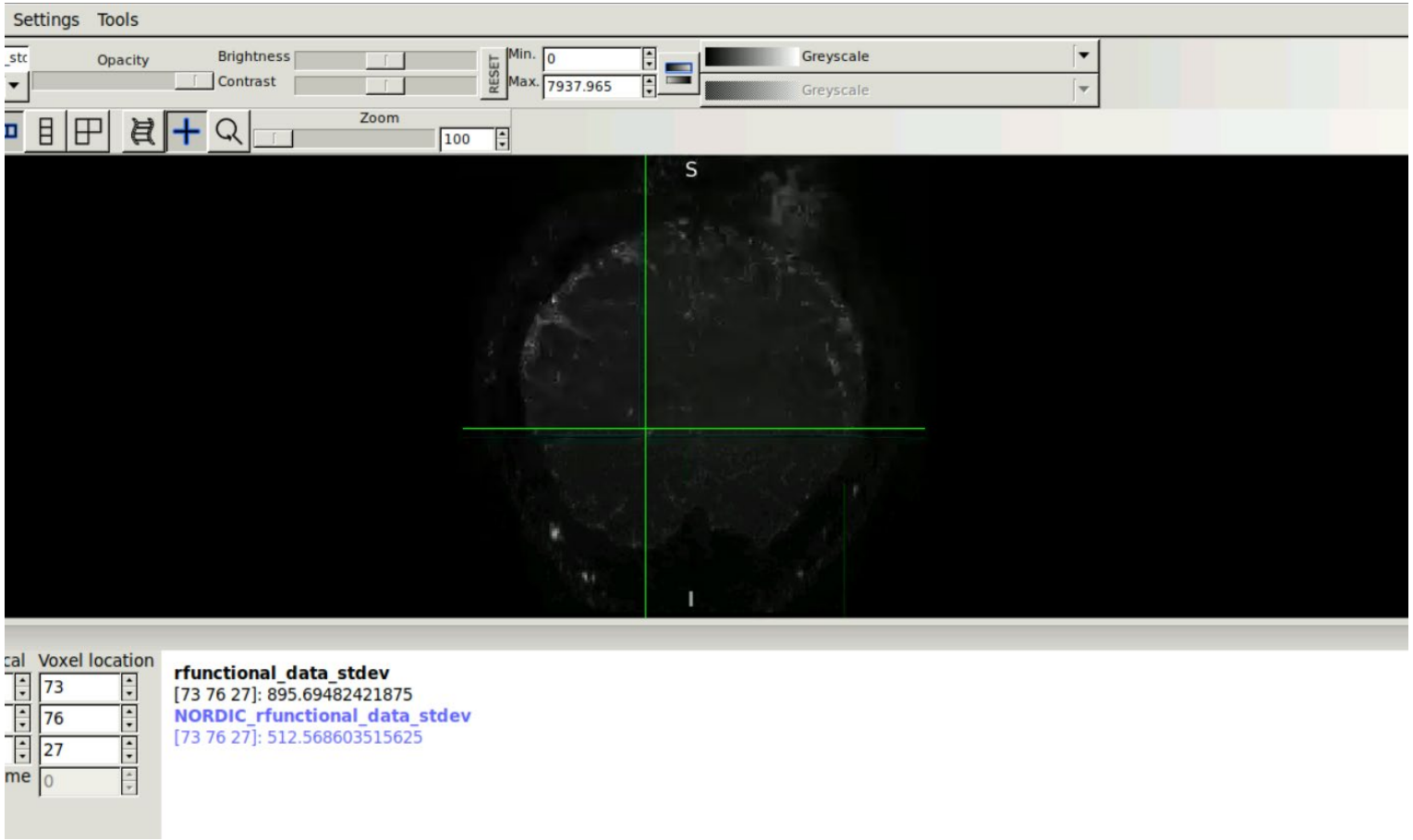
Quality Check (GLM result, no NORDIC, ONLY BOLD)

Lots of noise and false positives!



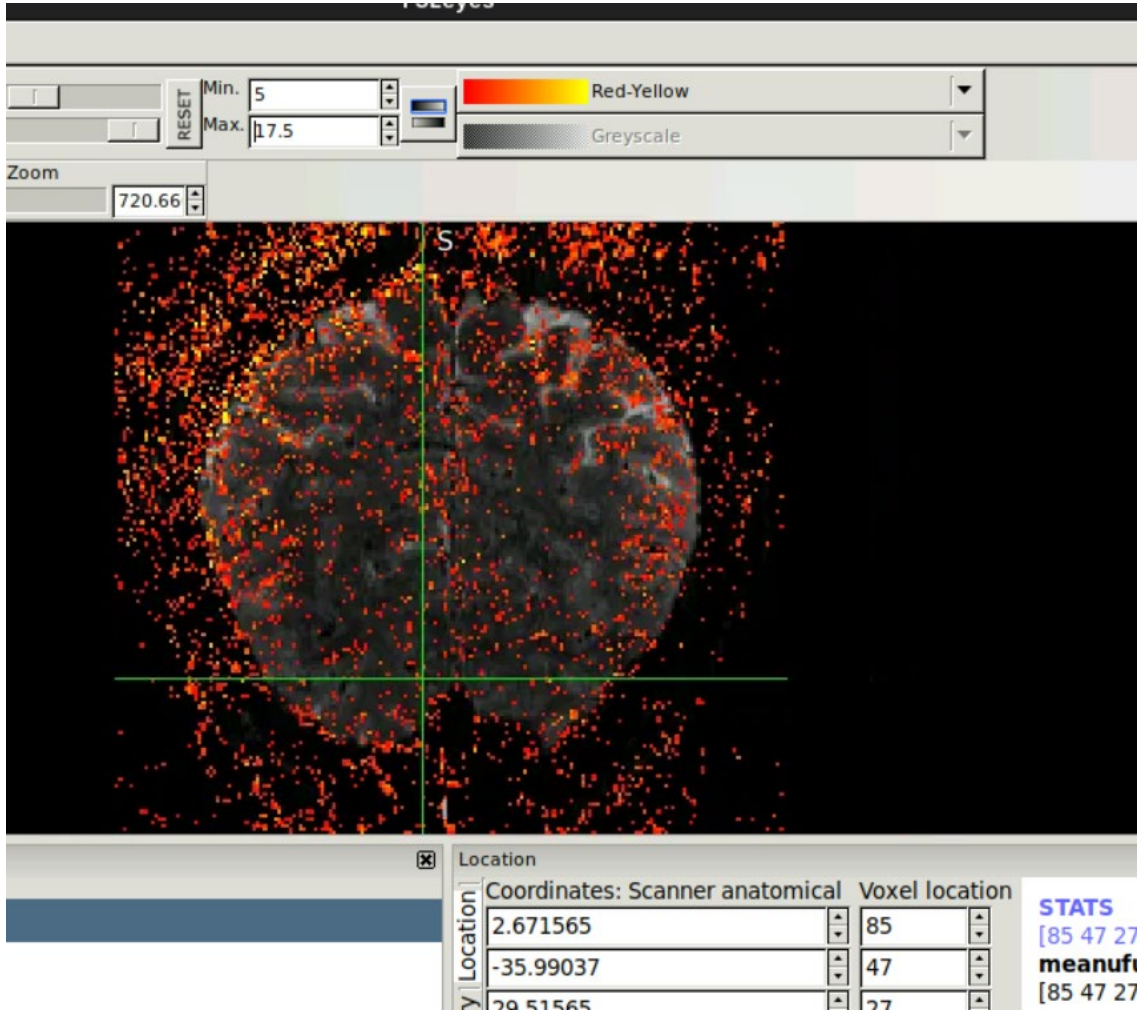
Quality Check (after NORDIC)

Overall std reduced and tSNR improved, but we have the ghost effect, yet.



Quality Check (after NORDIC)

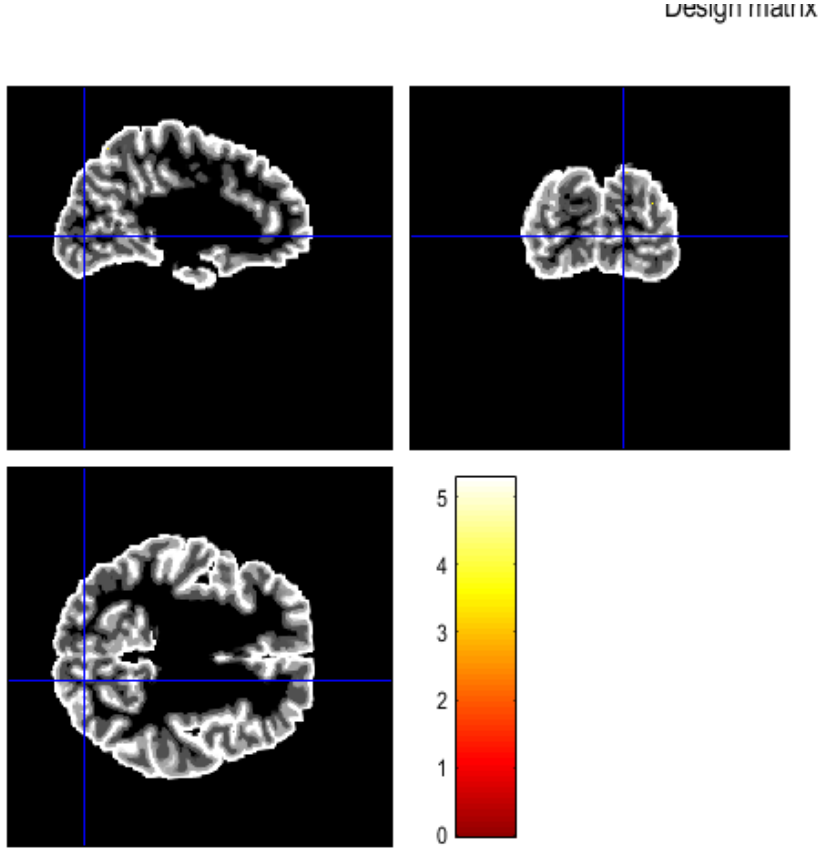
I don’t know why! But it seems the false positives increase!



GLM results and Layers in SPM

Ori2 – Ori1 (occipital mask, whole brain)
Then I used layersbin in overly section in SPM, to see whether I could see layer activation in this way! (I am a bit blind at this stage.. Found no good resources)

I couldn’t see activation in layers with SPM result.
Lets try with Afnii and FSLleys



Activation in v1 (upsampling and masking)

```
# Extract original voxel dimensions
x=$(3dinfo -di T1_weighted.nii)
y=$(3dinfo -dj T1_weighted.nii)
z=$(3dinfo -dk T1_weighted.nii)
# Calculate new voxel dimensions (e.g., upsample by a factor of 5 in x and y, keep z the same)
x=$(echo "((sqrt($delta_x * $delta_x) / 5))" | bc -l)
y=$(echo "((sqrt($delta_y * $delta_y) / 5))" | bc -l)
z=$(echo "((sqrt($delta_z * $delta_z) / 1))" | bc -l)
# Upsample the layersegmented image
3dresample -dxyz $x $y $z -rmode Cu -overwrite -prefix scaled_T1.nii -input ribbon-in-
rawavg_rim_layerbins_equivol.nii
# Upsample the functional image
3dresample -dxyz $x $y $z -rmode Cu -overwrite -prefix scaled_BOLD.nii –input NORDIC_rfunctional_data.nii
# Apply V1 mask to the upsampled anatomical image
3dcalc -a ribbon-in-rawavg_rim_layerbins_equivol.nii -b v1mask.nii -expr 'a*b' -overwrite -prefix
masked_scaled_T1.nii
# Apply V1 mask to the upsampled functional image
3dcalc -a NORDIC_rfunctional_data.nii -b v1mask.nii -expr 'a*b' -overwrite -prefix masked_scaled_BOLD.nii
```

Activation in v1 Layers

Code : `LN2_PROFILE -input masked_scaled_BOLD.nii -layers masked_scaled_T1.nii -plot -output layer_profile_cond1.txt`

Unfortunately, this doesn't works properly on this dataset, I guess due to poor co-registeration. Dr ke jia noted about the problem of co-registering this data, and I guess my co-registeration step was wrong! But the code works well in other datasets.

Final Note

Dear Prof. Peter,

During this short period, I believe I have gained a preliminary understanding of your layer-specific fMRI pipeline. While this does not represent my proficiency in analyzing fMRI data, as I am still new to this process, it does demonstrate that with the right resources and mentorship, I can learn quickly and contribute effectively to your lab.

Best,
Armin